Regulatory Toxicology and Pharmacology 89 (2017) 131–147

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

A retrospective analysis of *in vivo* eye irritation, skin irritation and skin sensitisation studies with agrochemical formulations: Setting the scene for development of alternative strategies

M. Corvaro [a, *], S. Gehen [b], K. Andrews [a], R. Chatfield [a], F. Macleod [a], J. Mehta [a]

[a] Dow AgroSciences Ltd - European Development Centre, Milton Park, Abingdon, OX14 4RN, UK
[b] Dow AgroSciences LLC - Headquarters, 9330 Zionsville Road, Indianapolis, IN 46268, USA

ARTICLE INFO

Article history:
Received 10 April 2017
Received in revised form
26 June 2017
Accepted 27 June 2017
Available online 28 June 2017

Keywords:
Skin irritation
Eye irritation
Delayed dermal sensitisation
GHS
Additivity formula
In vitro alternatives
Animal testing
Global policy change

ABSTRACT

Analysis of the prevalence of health effects in large scale databases is key in defining testing strategies within the context of Integrated Approaches on Testing and Assessment (IATA), and is relevant to drive policy changes in existing regulatory toxicology frameworks towards non-animal approaches. A retrospective analysis of existing results from *in vivo* skin irritation, eye irritation, and skin sensitisation studies on a database of 223 agrochemical formulations is herein published. For skin or eye effects, high prevalence of mild to non-irritant formulations (i.e. per GHS, CLP or EPA classification) would generally suggest a bottom-up approach. Severity of erythema or corneal opacity, for skinor eye effects respectively, were the key drivers for classification, consistent with existing literature. The reciprocal predictivity of skin *versus* eye irritation and the good negative predictivity of the GHS additivity calculation approach (>85%) provided valuable non-testing evidence for irritation endpoints. For dermal sensitisation, concordance on data from three different methods confirmed the high false negative rate for the Buehler method in this product class. These results have been reviewed together with existing literature on the use of *in vitro* alternatives for agrochemical formulations, to propose improvements to current regulatory strategies and to identify further research needs.

1. Introduction

Agrochemical formulations are end-use commercial products for professional and non-professional use as pest control agents. Assuming that a complete assessment for the agrochemical active substances is available, the registration process (i.e. to obtain authorisation to market the products) generally requires toxicological testing on the commercial mixture, usually limited to acute toxicity. As already analysed by the authors elsewhere (Corvaro et al., 2016), the key driver for characterising acute local toxicity properties of the end-use agrochemical product is to provide appropriate hazard communication via labelling.

In most geographies, the standard regulatory requirement is a set of six *in vivo* studies which examine systemic toxicity end-points for the primary potential exposure routes (oral, inhalation and dermal), as well as skin irritation, eye irritation, and skin sensitisation, also referred to as a "6-pack". Our previous research has been focused on a retrospective analysis of acute systemic toxicity properties and alternatives while, in this paper we will be focusing on the remaining 3 end-points which we will define as 'local toxicity' (we will use this definition for the three tests in question, although skin sensitisation has a systemic component).

The accepted regulatory tests are usually performed *in vivo*, using the concentrate product as is, which is a mixture of one or multiple active ingredients and a number of co-formulants (or inerts) mainly aimed to improve the efficacy of the active principle in the environment. The regulatory tests for skin and eye irritation end-points, are generally performed in Albino rabbits according to the method originally described by Draize in the 1940's (Draize et al., 1944) and subsequently ratified in OECD test guidelines

Abbreviations: ANVISA, Agência Nacional de Vigilância Sanitária (Brazil); AF, Additivity formula; EPA, Environmental Protection Agency (US); CLP, Classification and Labelling of Product, Regulation (EC) No. 1272/2008; GHS, Globally Harmonised System for classification and labelling; HSE, UK Health and Safety Executive; OECD, Organisation for Economic Co-operation and Development (Global); CO, Corneal opacity; Conj, Conjunctival effects; CC, Conjunctival chemosis; CR, Conjunctival redness; IR, Iris inflammation; E, Erythema; O, Oedema.

* Corresponding author.
E-mail address: mcorvaro@dow.com (M. Corvaro).

http://dx.doi.org/10.1016/j.yrtph.2017.06.014
0273-2300/

(TG) no. 404 and 405 (more recent versions are OECD, 2002; OECD, 2012b). From an animal use perspective, these tests have not been formally "validated" or, in other words, evaluated for their ability to predict the irritation potential to humans. In fact, some aspects of their validity have been questioned in the last 10–15 years (See in example Scott et al., 2010; Adriaens et al., 2014; Barroso et al., 2016). From an animal welfare perspective, they certainly can induce discomfort to animals in certain cases, particularly for the eye where the use of anaesthetic has been introduced in the more recent version of the OECD TG. For dermal delayed sensitisation there are multiple *in vivo* tests currently accepted in most geographies. The "old generation" test in guinea pigs (with two methods, Buehler and Magnusson and Kligman; described in OECD TG 406; OECD, 1992) have never been validated for human predictivity (Basketter and Kimber, 2010). The final interpretation of the study is based on the detection of an *in vivo* apical adverse effect following an initial sensitisation phase (induction) using the maximum slightly irritating concentration and a challenge (sensitisation reaction) with a non-irritant concentration, with a potentially severe and painful allergic reaction. These aspects together with the use of adjuvants and occlusive dressing raised concern from an animal use and welfare perspective. Today, the more modern local lymph node assay (LLNA; OECD, 2010a), is preferred to the guinea pig test as it underwent a formal validation. In addition, focusing on the induction phase, it reduces animal use, pain and distress. However, the LLNA is not without limitations in terms of biological variability, human predictivity (false positives and negatives rates), can show variability in potency prediction and, notably, validation did not include the agrochemical formulations (Basketter and Kimber, 2009, 2011: Kolle et al., 2013a; Hoffmann, 2015; Roberts et al., 2016a,b,c; Leontaridou et al., 2017).

Recently developed regulatory frameworks have seen considerable changes in the strategy used for hazard characterisation of 'local toxicity', both for single chemicals and mixtures. In fact, use of alternative methods (*i.e.* non-testing, read across, *in silico* or *in vitro*) was introduced in the pharmaceutical sector (Roberts and Patlewicz, 2010; Goh et al., 2015) and, in a triggered fashion, in the chemical sectors (*i.e.* particularly with the 'REACH' regulation; EU, 2006). However, in some areas, a complete removal of *in vivo* testing was implemented, as in the cosmetic sector where the new EU regulatory framework has impacted on the actual testing feasibility for both ingredients and end-use products on a global scale (EU, 2009b). Little advancement has been made in the regulatory requirements for agrochemical formulations and a lack of global harmonisation in requirements is an issue for the real implementation of alternative strategies (Table 1). The regulation EU 1107/2009 and the connected regulation EU 283/2013 on data requirements (EU, 2009a; 2013b) have introduced the legal requirement to use alternative approaches where these are suitable for hazard identification and Classification and Labelling, referring back to the horizontal CLP regulation and test method regulation EU 1272/2008 and EU 440/2008 (EU, 2008b; 2008a), respectively. In a note of clarification from the UK Health and Safety Executive (UK HSE (2017)), currently part of the European Union, it is clarified that the calculation approach proposed by GHS (UN, 2015a) must be used together with suitable *in vitro* alternatives, where appropriate. Australia and New Zealand do accept read-across, calculation methods and encourage the use of OECD *in vitro* methods as alternative to *in vivo* tests (APVMA, 2015; NZ EPA, 2016). In Canada and US, there is currently a limited possibility of bridging from existing data on similar formulations (EPA, 2012a; PMRA, 2013). The US EPA has implemented a pilot program requesting parallel submission of *in vitro*/calculation approaches and *in vivo* results to support future policies with greater acceptance of alternative methods (US EPA, 2015; 2016b). In Brazil, the national council for the control of animal experiments, (CONCEA) part of the Ministry of Science, Technology and Innovation has issued a resolution document listing all accepted alternatives to animal testing in 2014 (CONCEA, 2014). However, these were not all implemented in the new draft regulation for pesticide approval (ANVISA, 2015; in commenting phase at the time of this publication) where only *in vivo* testing is acceptable for classification according to the old regulation (ANVISA, 2011). Requirements in other geographies (most of the Asian and Latin American countries) do not currently include the possibility of using calculation or *in vitro* methods as first choice methods.

To facilitate regulatory discussion and promote harmonisation in member and non-member countries, the OECD has been developing frameworks called IATA (Integrated Approaches to Testing and Assessment (IATA) (OECD, 2008), defined as "pragmatic, science-based approaches for chemical hazard characterisation that rely on an integrated analysis of existing information coupled with the generation of new information using testing strategies". These are intended to consider all available methodology relevant for each endpoint, characterising the predictivity and uncertainty related to each method and describing how they can be used in an overall scientific weight of evidence. A key starting point of IATA is to characterise the toxicity features of a certain chemical space, in order to understand the prevalence of certain findings and define the best (testing/non-testing) strategy. Another aspect is to elaborate on the predictivity of alternative approaches, as well as the uncertainties related to the prediction of the human outcome, based on available data with alternative approaches. This information will help define the best approach to build a weight of

Table 1
Lack of global harmonisation in standard and alternative requirements for eye and skin irritation.

	in vivo tests	*in vitro* tests	Read Across from similar formulations with an *in vivo* test	GHS additivity
Australia/New Zealand	Accepted	Accepted	Possible	Accepted
EU	Accepted (only option for some member states)	Accepted, if conclusive	Possible, but acceptability is discretionary	Possible, but acceptability is discretionary
USA	Main requirement	Pilot program on going for future acceptability	Possible	Pilot program on going for systemic toxicity
Canada	Accepted	Pilot program on going for future acceptability	Possible	Pilot program on going for systemic toxicity
Brazil	Accepted	Not accepted, some discussions ongoing	Possible	Not accepted
Other latin-america countries	Accepted	Not accepted	Possible, in some countries	Not accepted
Asian countries	Accepted	Not accepted	Generally, not accepted	Not accepted